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Survey of the Sensitivity of Microorganisms to Aflatoxin

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ABSTRACT

BURMEISTER, H. R. (Northern Regional Research Laboratory, Peoria, Ill.), AND C. W. HESSELTINE. Survey of the sensitivity of microorganisms to aflatoxin. Appl. Microbiol. **14**:403–404. 1966.—Among the 329 microorganisms tested for aflatoxin sensitivity were 30 genera of bacteria, 34 genera of fungi, 4 genera of algae, and 1 protozoan. Twelve species of the genus *Bacillus*, a clostridium, and a streptomycete were inhibited when 30 μg/ml of crude aflatoxin (36% pure) was incorporated into the growth substrate. A strain of *Bacillus brevis* and two of *B. megaterium* were most sensitive to aflatoxin, being inhibited at 10 and 15 μg/ml, respectively.

Aflatoxin-sensitive microorganisms may serve a number of purposes in expanding existing knowledge of this metabolite. For example, the mechanism of toxicity in microorganisms might be studied. Tests employing microbes might supplement the duckling assay in establishing toxicity levels of aflatoxin in contaminated feeds. Lastly, aflatoxin-sensitive microorganisms could be employed in an assay where toxin levels are in excess of that required to inhibit the test organism.

MATERIALS AND METHODS

Aflatoxins used in this study were produced by *Aspergillus flavus* Link ex Fries NRRL 2999 grown on rice. The aflatoxins were extracted from the rice with chloroform and precipitated with hexane. The hexane precipitate contained, by weight, 23.8% B_1, 6.3% B_2, 6.8% G_1, and 0.9% G_2 as determined by thin-layer chromatography. Portions of a chloroform solution of the precipitate were dispensed into bottles, and the chloroform was driven off in a steam chamber before adding medium and autoclaving. Concentrations of crude aflatoxin used for the general screening were 0, 5, 10, 15, 20, and 30 μg/ml of medium. A 5-μg amount of the crude aflatoxin contained the following amounts of the pure toxins: 1.19 μg of B_1, 0.31 μg of B_2, 0.34 μg of G_1, and 0.045 μg of G_2.

The medium employed for culturing the actinomycetes was a modification of that of Warren, Prokop, and Grundy (3); i.e., 0.1% yeast extract was substituted for the 0.5% Curbay BG. Strains of *Bacillus popilliae* were cultured on the medium described by St. Julian, Pridham, and Hall (2). Clostridia, lactobacilli, and streptococci were inoculated as stabs into tubes of deep liver-agar; acetobacter agar was the growth substrate for *Acetobacter* sp.; and MY broth was used to test the protozoan. Algae, yeasts, molds, and all bacteria not previously mentioned were cultured as surface streaks on tryptone-glucose-yeast extract-agar (TGY). These media were described by Haynes, Wickerham, and Hesseltine (1).

Paper chromatograms of the crude aflatoxin precipitate were developed with two solvent systems by ascending technique on Whatman no. 1 paper: (i) chloroform plus 3% methanol and (ii) water with 20% methanol and 1% formic acid. The aflatoxins were located on the paper chromatogram with ultraviolet light, and the fluorescent zones were marked before the biological test was made. A microbiological test was conducted with a spore suspension of *B. megaterium* NRRL B-1368. Medium TGY with 1% agar, adjusted to *p*H 6.2, was seeded with 1% of a suspension containing 3×10^7 spores per milliliter and then was poured into chromatographic trays. After drying, the developed chromatograms were placed on the surface of the inoculated-agar medium and incubated for 18 hr at 30 C. The resulting bioautographs had zones of inhibition corresponding to the fluorescent zones on the paper strips; no other zones of inhibition were noted.

RESULTS AND DISCUSSION

Microorganisms found sensitive to the aflatoxins, at the levels tested, are restricted to strains of gram-positive, spore-forming bacilli and to one streptomycete. A list of sensitive bacteria is presented in Table 1 and a list of nonsensitive microorganisms in Table 2. *B. brevis* NRRL B-1874 and two strains of *B. megaterium*, NRRL B-1368 and NRRL B-1370, are inhibited by 15 μg/ml of crude aflatoxin or less. Twelve strains of 19 species of the genus *Bacillus* were sensitive to 30

TABLE 1. *Aflatoxin-sensitive microorganisms*

Microorganism	No. of strains	Lowest concn of crude aflatoxin* inhibiting growth
		μg/ml
Bacillus megaterium	1	5
B. brevis	1	10
B. megaterium	2	15
B. megaterium	3	20
B. licheniformis	1	20
B. sphaericus	1	20
B. subtilis	1	20
B. thuringiensis	1	20
B. alvei	1	30
B. bombysis	1	30
B. cereus	5	30
B. licheniformis	3	30
B. macerans	2	30
B. pumilus	1	30
B. subtilis	3	30
B. technicus	1	30
B. cereus	2	40
B. thuringiensis	2	40
Clostridium sporogenes	1	30
Streptomyces sp. F-2672	1	20

* The crude aflatoxin precipitate contained a total of 36% pure aflatoxin.

μg/ml of the crude precipitate. Inhibition varied among strains of the same species.

One strain of *Clostridium sporogenes* and 1 of 80 streptomycetes tested were inhibited when 30 μg/ml of crude aflatoxin was incorporated into the growth substrate. No inhibition was noted in 11 genera of gram-positive and 16 genera of gram-negative bacteria. All the eucaryotic microorganisms tested were visibly unaffected by aflatoxin.

TABLE 2. *Microorganisms not inhibited by 30 μg/ml of aflatoxin**

Taxon	No. of genera	No. of species
Pseudomonadales	5	26
Eubacteriales		
Rhizobiaceae	1	1
Micrococcaceae	3	3
Lactobacteraceae	4	12
Achromobacteraceae	3	6
Enterobacteriaceae	5	7
Corynebacteriaceae	1	3
Bacillaceae	2	18
Hyphomicrobiales	1	1
Actinomycetales	3	14
Yeasts	16	38
Other fungi	18	25
Algae	4	—
Protozoa	1	1

* The crude aflatoxin precipitate contained a total of 36% pure aflatoxin.

The yeasts were not inhibited by concentrations of 40 μg/ml of the crude aflatoxin.

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LITERATURE CITED

1. HAYNES, W. C., L. J. WICKERHAM, AND C. W. HESSELTINE. 1955. Appl. Microbiol. 3:361–368.
2. ST. JULIAN, G., JR., T. G. PRIDHAM, AND H. H. HALL. 1963. J. Insect Pathol. 5:440–450.
3. WARREN, H. B., JR., J. F. PROKOP, AND W. E. GRUNDY. 1955. Antibiot. Chemotherapy 5:6–12.